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Sources of Psychrophilic Bacteria in a Dairy Plant With an Emphasis on Air

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To the Graduate Council:

I am submitting herewith a thesis written by Kotha Madhava Rao entitled "Sources of Psychrophilic Bacteria in a Dairy Plant With an Emphasis on Air." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

W. W. Overcast, Major Professor

We have read this thesis and recommend its acceptance:

J. T. Miles, B. J. Dermott, R. S. Dotson

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

December 14, 1965

To the Graduate Council:

I am submitting herewith a thesis written by Kotha Madhava Rao entitled "Sources of Psychrophilic Bacteria in a Dairy Plant With an Emphasis on Air." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairy Manufacturing.

W W Overcast
Major Professor

We have read this thesis and recommend its acceptance:

J. T. Miles

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Accepted for the Council:

Hilton A. Smith
Dean of the Graduate School

SOURCES OF PSYCHROPHILIC BACTERIA IN A DAIRY PLANT WITH AN EMPHASIS ON AIR

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Kotha Madhava Rao

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TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

CHAPTER I

INTRODUCTION

The dairy industry generally recognizes that the psychrophilic bacteria are those bacteria which can grow in milk and milk by-products at refrigerated temperatures. Although psychrophiles have been recognized since the latter part of the last century, they were of relatively little importance in the dairy industry, as long as the milk and milk products were consumed within a reasonably short period of time after production or processing. The presence of psychrophilic bacteria in milk has been attracting increasingly more wide spread attention since the early 1950's because of the introduction of bulk tanks on the farm, the related every other day pick-up and delivery, and subsequent storage prior to use. Because of these and related changes the dairy industry requires high quality milk that will maintain this quality for an extended period at refrigeration temperatures.

The presence of certain types of psychrophilic bacteria in milk causes various off flavors and odors; this

lowers quality and value. The present study was made in order to determine the various sources of psychrophilic bacteria in a dairy plant with special reference to air as one of the possible contaminating sources. Research data of this type may be of value to processors and quality control personnel of the industry.

CHAPTER II

REVIEW OF LITERATURE

I. DEFINITIONS

The term psychrophilic bacteria means (Greek origin; psychro--cold, philo--loving) cold loving bacteria. The first recorded knowledge of bacteria which grow at low temperatures was credited to Forester, 1887 (37).[1] Schmidt-Nielson coined the term, psychrophile in 1902 to describe the bacteria which grow at low temperatures (37). Actually these organisms are cold tolerant rather than cold loving since their optimum temperature for growth may be 20° C. or above (9). Some attempts have been made to change the terminology but these have not been successful. Some other terms used in the literature are as follows, psychrocartericus or cold conguering, glaciale, eurythermic, thermophobic, psychrobe, righopiles (37), cryophiles (37, 39, 81), and psychrotolerant (37, 81). No absolute agree-

[1] Numbers in parentheses refer to Literature Cited.

ment for the definition of psychrophiles and their growth temperatures occurs in the literature. Standard Methods for the Examination of Dairy Products (3) defines psychrophilic bacteria as ". . . those which are capable of relatively rapid growth at low temperatures, commonly within the range of 35 to 50° F."

Olson et al. (55) pin-pointed this common range as 35 to 45° F. Thomas (70) concluded that there was general agreement that psychrophiles were able to grow at temperatures below 5° C. and do not survive heating in milk at 63° C. for thirty minutes. Kennedy and Weiser (41) defined psychrophilic bacteria as the organsims which have an optimum temperature range between 5 and 25° C. Witter (81) observed that the psychrophilic bacteria grow relatively rapidly below 45° F. Ingraham (36) stated that the ability to make relatively rapid growth at low temperatures was the distinguishing characteristic of psychrophiles, and further stated that the generation time must be less than forty-eight hours at 0° C. Ingraham and Stokes (37) defined the psychrophilic bacteria as those that form easily visible colonies on solid media within two weeks at 0° C. Foster et al. (27) defined the psychrophilic microorganisms as

". . . those which are able to develop comparatively rapidly at temperatures below 15° C. (59° F.)." Schultze and Olson (65) considered the psychrophilic bacteria as those which have the ability to multiply sufficiently rapidly to be a significant contribution to the microflora at a given low temperature. Zobell and Conn (82) after studying the thermal sensitivity of marine bacteria, stated that true psychrophiles were not found in spite of the fact that marine bacteria grew slowly at near zero temperatures.

A logical deduction can be made from the viewpoints of several authors that the psychrophilic bacteria are those bacteria which grow relatively rapidly at low temperatures. Perhaps due to this agreement the dairy industry, in general, considers the low temperatures as refrigeration temperatures. This then has a practical bearing beyond that of classical importance.

II. GROWTH TEMPERATURES

Minimum temperature. Berry and Magoon (9) stated that any microbial growth below minus 10° C. seems to be unlikely. Thomas (70) noted that the minimum range of growth temperatures is minus 5 to 0° C. Ingram (35)

observed the slow growth of halophilic bacteria on bacon at minus 10° C. However, Witter (81) stated that the growth of bacteria below minus 10° C. was unlikely.

Optimum temperature. Thomas (70) pointed out the optimum temperature range as 10 to 20° C. for psychrophiles. Ingraham and Stokes (37) stated the optimum temperature was the temperature where the rate of growth was most rapid and further that for psychrophilic bacteria this temperature was near 20° C. or above 20° C. (20 to 40° C.). Zobell and Conn (82) observed that the optimum temperature was within the range of 18 to 22° C. This was based on maximum colony counts after one to two weeks of incubation. When all authorities were considered, 20° C. appeared to be most often considered as an optimum temperature.

Maximum temperature. Thomas (70) considered 25 to 35° C. as the range of maximum growth temperature. Ingraham and Stokes (37) pointed out that most strains have a maximum growth temperature of 30° C. although there were organisms with a maximum growth range of from 37 to 45° C. Witter (81) reported that most text books suggest a maximum growth temperature for psychrophilic bacteria of 30° C.

III. ENUMERATION METHODS

Schultze and Olson (64), Olson *et al.* (55), and Thomas *et al.* (74), incubated agar plates for ten days at 7° C. for psychrophilic colony counts. After making a comparative study of psychrophilic counts at 3 to 5° C. and at 7 ± 0.5° C. Thomas et al. (73) recommended incubation of agar plates for ten days at 7 ± 0.5° C. *Standard Methods for the Examination of Dairy Products* (3) recommends the incubation of plates at 5 to 7° C. for seven to ten days. Jezeski and Macy (39) reported that the incubation of plates at 8° C. for seven days gave higher counts than incubation at higher temperatures like 37° C. However, in most cases highest counts were obtained at 20° C. Greene and Jezeski (31) incubated the plates at 20° C. for five days in a study related to the influence of temperature on the development of various psychrophilic bacteria of dairy origin.

IV. TYPES OF PSYCHROPHILIC BACTERIA

Perhaps the organisms belonging to genus *Pseudomonas* are the most commonly encountered type of psychrophiles (16, 20, 29, 39, 48, 50, 55, 64, 65, 72, 81). Thomas and Druce

(72) summarized the results obtained by various workers both in raw and pasteurized milk and listed the organisms as belonging to genera Pseudomonas, Achromobacter, Alcaligenes, Flavobacterium, Arthrobacter, Micrococcus, Chromobacterium besides coli-aerogenes organisms. The same authors stated that the organisms belonging to the genera Pseudomonas or Achromobacter were generally predominant, the species of genus Flavobacterium were occasionally dominant, and the organisms belonging to genus Alcaligenes and coli-aerogenes organisms formed a small proportion of psychrophilic bacteria. After studying 235 cultures isolated from the refrigerated raw milk these authors stated that 94 per cent of these cultures were fluorescent pseudomonads and non-fluorescent pseudomonads or "achromobacteria." Andrey and Frazier (6) isolated 220 cultures from twelve farm bulk cooling tanks in Wisconsin and found the organisms belonged to the genera Aerobacter, Alcaligenes, Arthrobacter, Flavobacterium, Micrococcus and Pseudomonas. These research workers found that the organisms of the genus Arthrobacter were predominant in the milk when the cows were barn fed, while the Flavobacterium species were predominant when the cows were fed on pasture.

Jezeski and Macy (39) isolated forty-one cultures from water samples obtained from Minnesota and Wisconsin creameries which had been experiencing difficulty with keeping quality of butter. These workers found twenty-eight cultures were Pseudomonas species and twenty-one of them produced green fluorescence in culture media. Five cultures were yellow pigment-producing Flavobacterium species, six were placed in the genus Achromobacter, one was in the genus Alcaligenes, while the last one was a non-lactose fermenting yeast. Jezeski (38) observed that the organisms of Pseudomonas, Alcaligenes, and Achromobacter species affect the keeping quality of dairy products. Olson et. al. (55) reported that the psychrophilic bacteria are largely gram-negative rods and commonly belong to genera Pseudomonas, Flavobacterium, Proteus, Alcaligenes, and Achromobacter in addition to certain coliforms. Thomas and Sekhar (77) as cited by Thomas (70), found only three gram-positive yellow pigment-producing micrococci out of 206 strains of psychrophilic bacteria isolated at 3 to 5° C. for twenty-one days. The remaining 203 strains were gram-negative rods, the majority of which were Achromobacter species and some non-chromogenic strains might have belonged to the genus Alcaligenes.

Corley et al. (16) examined 436 water samples from water supplies of seventy butter plants in Iowa and found that 24 per cent contained typical fluorescent colonies resembling Pseudomonas fluorescens or closely related organisms, 5 per cent contained Pseudomonas putrefaciens, while some other isolations were Pseudomonas graveolens, Pseudomonas fragi and Pseudomonas mephitica. Brown and Weidemann (13), after reassessing the taxonomy of meat spoilage bacteria, reported 182 gram-negative rods out of 185 psychrophilic bacteria isolated from chilled beef, and further stated that 128 out of 182 gram-negative rods were typical Pseudomonas species with polar flagella. Marth and Frazier (48) after studying the bacteriology of raw milk held at farm bulk cooling tank temperatures observed that the organisms were gram-negative rods belonging to the genera Achromobacter, Aerobacter, Alcaligenes, Flavobacterium, and Pseudomonas. These organisms were isolated from raw milk and then grown at 38° F. for four days, and the authors noticed most rapid growth by Pseudomonas cultures followed by cultures of Achromobacter, Alcaligenes, other Pseudomonas, and Aerobacter; while Flavobacterium cultures failed to grow appreciably. Davis (20) stated that

the commonly encountered psychrophilic organisms belong to genera Pseudomonas, Achromobacter, Chromobacterium, and Micrococcus.

Schultze and Olson (64) isolated 620 cultures from commercially packaged milk, cream, chocolate drink, and cottage cheese stored at 4° C. for one week during summer and winter. The plates were incubated at 7° C. for ten days. The taxonomic study indicated that these organisms belonged to genera Pseudomonas, Alcaligenes, Achromobacter, Flavobacterium, members of atypical coliform groups which fermented lactose very slowly without gas production, and a few yeasts. Over one-half of these 620 isolates were fluorescent pseudomonads. In another study, Schultze and Olson (65) isolated 586 cultures from pasteurized samples of milk, cream, chocolate drink, and cottage cheese after storage at 4° C. for seven days (excepting the cottage cheese which was stored at 4° C. for fourteen days). The characterization of the organisms showed that 70.6 per cent were Pseudomonas species, 7.9 per cent were Alcaligenes species, 9.2 per cent were Achromobacter species, 0.7 per cent were Flavobacterium species, while 10.8 per cent were coliforms and the remaining 0.8 per cent were yeasts.

Witter (81) stated that some organisms belonging to genera Pseudomonas, Achromobacter, Alcaligenes, Flavobacterium, Escherichia, Aerobacter, Serratia, Proteus, Chromobacterium, Arthrobacter, Lactobacillus, Streptococcus, Micrococcus, Bacillus, and Sarcina were psychrophilic bacteria.

Erdman and Thornton (22) isolated 722 cultures of psychrophilic bacteria from milk and cream and incubated the plates at 4.5 or 10.5° C. for seven days. Erdman and Thornton (23) further observed out of 722 cultures, 45 per cent were rods and 55 per cent were coccal forms. Seventy per cent of the rods and, surprisingly, 62 per cent of the cocci were gram-negative. Coccal forms were predominant among gram-positive organisms. Among the 722 cultures, 26 per cent produced fluorescence. However, Thomas (70) stated some mesophiles were included as a result of incubation of the plates at 10° C. and this was responsible for a smaller number (31 per cent) of gram-negative rods and larger number (69 per cent) of gram-positive rods and cocci and gram-negative cocci. Thomas (70) further observed that Erdman and Thornton (23) did not encounter any spore formers. Rogick and Burgwald (63) isolated 167 psychrophilic cultures from milk by incubating plates at 4 to 7° C. for twelve days

and found 63.45 per cent were cocci and only 36.52 per cent were bacilli.

While the above mentioned types were most commonly mentioned, occasionally different species also were reported. Dahlberg (19) observed the growth of coliform bacteria in pasteurized milk after storage for none to four days at 35 to 40° F. and 45 to 50° F. Further, this work revealed the more rapid growth of coliforms than the total counts at 45 to 50° F. After storing the pasteurized milk at 45 to 50° F. the coliform bacteria represented 5.55 per cent of the total count in October and 88 per cent in July and August. At all three temperature ranges (35 to 45° F., 45 to 50° F., 55 to 60° F.), the increase of coliform bacteria was more rapid in warm summer months than during the cool month of October. Foter and Rahn (28) observed the growth of Streptococcus faecalis and Streptococcus glyceranicus at 0° C., but no explanation for growth at this low temperature was offered by the authors. After studying the growth and fermentation of Streptococcus glyceranicus, Streptococcus faecalis, Streptococcus liquefaciens, Streptococcus lactis, Lactobacillus acidophilus, the authors stated that lactic acid fermentation takes place even at low

temperatures like C. when large amount of inoculum was used.

Abd-El-Malek and Gibson (1) isolated Alcaligenes tolerans from dairy equipment and pasteurized milk. This organism could grow at 10° C. and at 37° C. with an optimum temperature of 30° C. (growth variable at 40° C.). Olson et al. (55) noted that the reports have indicated that predominant organisms also may vary with geographical area involved.

V. EFFECT OF PASTEURIZATION

Most available evidence (5, 7, 38, 58, 63, 67, 71, 72, 80) indicates that psychrophilic bacteria do not survive pasteurization. Watrous et al. (80) observed that psychrophilic bacteria were destroyed by pasteurization at 62.8° C. for thirty minutes. Andrews and Kaufmann (5) isolated sixty-six psychrophilic cultures from milk and water supplies. None of these survived pasteurization by high-temperature short-time method or at 143° F. for twenty-five minutes. This observation was based on 99.999 per cent destruction. Thomas and Druce (72) studied 235 psychrophilic cultures and none of them survived laboratory

pasteurization in milk at 63.5° C. for thirty minutes. None of the Pseudomonas organisms of the 199 green fluorescent organisms isolated by Seleen and Stark (67) survived heating for thirty minutes at 60° C. Rogick and Burgwald (63) could not detect any psychrophilic bacteria in 4.1 milliliter of milk from samples taken in sterile bottles directly from the vat or holding tubes.

Only 4 out of 722 psychrophilic isolates of Erdman and Thornton (23) survived laboratory pasteurization at 142° F. for thirty minutes. These four were gram-negative, non-fluorescent cocci which did not liquify gelatin. Jezeski and Macy (39) noted the survival of only six out of forty-one cultures when heated at 150° F. for thirty minutes, and these surviving organisms showed considerable caseolytic activity.

Kennedy and Weiser (41) isolated fifteen pure cultures of psychrophilic bacteria from raw milk and fourteen of them survived laboratory pasteurization at 145° F. for thirty minutes. However, these research workers added that in seven cultures the organisms were reduced by 90 per cent and over, in five cultures the reduction in number of organisms was from 50 to 90 per cent, one culture had

24 per cent reduction, while one culture had only 4.8 per cent reduction in number of organisms. A bacterial count before and after pasteurization was made by incubating the plates at 10° C. for two days. The count ranged from 4,500 to 560,000 per milliliter before pasteurization and from 0 to 120,000 per milliliter after pasteurization. Alcaligenes tolerans isolated by Abd-El-Malek and Gibson (1) survived pasteurization at 63° C. for thirty minutes. Rogick and Burgwald (63) stated that the psychrophilic bacteria were invariably present in raw milk, but none survived pasteurization. However on examination of the samples after storing at refrigerated temperatures, the authors found some psychrophiles and concluded that all psychrophilic bacteria were not destroyed but the surviving bacteria were so few and hence could not be detected in the amounts of milk tested. The same authors further stated that some mesophiles developed psychrophilic tendencies.

At the end of the review of the available literature on this topic a general deduction could be made that the psychrophilic bacteria which survive pasteurization were non-pathogenic and their numbers, if any, were of no

consequence to keeping quality of milk provided the milk was not exposed to post-pasteurization contamination and was consumed in a reasonably short time.

VI. SOURCES

The natural sources of psychrophilic bacteria are soil and water (40, 70, 81). Some of the pre-pasteurization sources of psychrophilic bacteria are bedding of cows, the flanks, and exterior of udder (70). Water from farm, lakes, streams, and dairy plant supply units also contribute to pre-pasteurization contamination (81).

VII. POST-PASTEURIZATION SOURCES

Water. Corley et al. (16) detected psychrophilic bacteria from 127 samples out of 436 samples examined from water supplies of seventy butter plants. Jezeski and Macy (39) plated twelve water samples and incubated the plates at 8° C. for seven days. In three instances, the colony counts exceeded one thousand per milliliter, while the tests for coli-aerogenes organisms were always negative. Thomas and Thomas (78), as cited by Thomas (70), examined 126 water supplies in Wales and found only 3 per cent of the samples

had psychrophilic counts under ten per milliliter, while 70 per cent had counts over one hundred per milliliter and 14 per cent had counts of more than ten thousand per milliliter. Johns (40) observed that the usual treatment of water supplies killed coliforms but not psychrophiles, and the equipment coming in contact with such water was contaminated by the latter bacteria. Olson et al. (55) also observed that a treated water supply, otherwise satisfactory, may be a major source of psychrophilic bacteria.

Dairy equipment. Erdman and Thornton (22) attributed the non-sterile dairy equipment as the main source of the majority of psychrophiles in freshly pasteurized milk. These authors suggested the possibility of using psychrophilic counts as a measure of equipment sanitation and suggested further investigation in this matter. Thomas (70), after examining the data of Thomas and Rowland (76) stated that three kinds of conditions regarding the bacterial content of washed milk cans were to be:

> . . . (i) reasonably sterile cans relatively free from organisms of psychrophilic, mesophilic, or thermoduric type, (ii) presence of large numbers of thermodurics and few psychrophils and (iii) presence of large numbers of bacteria of all types.

Thomas et al. (74) determined the psychrophilic bacterial content of rinses of one hundred washed milk cans taken at five creameries by counting the colonies on yeastrel milk agar incubated at 7° C. for ten days. The results showed 70 per cent of the cans can act as source of psychrophilic bacterial contamination even though to a small degree. These same authors concluded that the detection of more than one million heat labile psychrophiles from the rinse of a milk can taken within half an hour after washing in a can washing machine was an indication of poor operation of the can washing machine. Thomas et al. (75) tested 387 quarter strength Ringer's solution rinses from dairy equipment from about one hundred farms. Each item was rinsed twice with 500 milliliters of this solution containing appropriate inactivators for chlorine and quaternary ammonium compounds. The rinse solutions were plated on yeastrel milk agar and incubated at 7° C. for ten days. Rinses from pipeline milking units gave higher colony counts than those of milking machine clusters, milking machine pails and milk cooling units. This was explained because steam was used for sterilization of milking machine clusters, pails, and milk cooling units. About 7 per cent of the rinses

contained over one million psychrophiles per square foot while the majority of the 387 rinses did not contain a large number of psychrophiles. Marth et al. (49) stated that flat open surfaces of plant equipment had usually been cleaned satisfactorily, but valves were not maintained in satisfactory condition by routine cleaning and required special care in cleaning and sanitizing. This statement was made by the authors on the basis of the results obtained from eight square inch areas of the plant equipment.

Rogick and Burgwald (63) reported that the bottles were a serious factor in contamination and found that fifteen out of thirty-four freshly washed half-pint bottles contained psychrophilic bacteria.

Air. Thomas (70) concluded that the air could not be considered as a serious contaminating source of psychrophilic bacteria. Fabian (25) conducted a study to determine the number of bacteria and molds in the air of an ice cream plant for a period of one year. In this study the samples were collected by using Ruehle type of aeroscope and by exposing ten-milliliter portions of sterile saline solution in sterile deep culture dishes, to the air for five minutes

and thereafter plating. First the standard agar plates were incubated at room temperature for five days and counted and then reincubated at 37° C. for two days. The plates made from milk powder agar were incubated for two days at 37° C. and then at room temperature for five days. The author concluded that the bacterial contamination from air was small and the number of organisms varied throughout the year, the largest number of bacteria being found during April, while the smallest was found during January. When other things were equal, weather was found to be the most important single factor influencing the number of micro-organisms in air in this experiment. The majority of organisms found by Fabian (25) were peptonizers, alkali-producing inert bacteria and a few weak acid-producing bacteria. The same study also revealed that the number of bacteria found was greater than the number of molds. Olson and Hammer (53) exposed malt agar plates and beef infusion agar plates inside and outside butter churns and said that the number of bacteria was higher than the number of yeasts or molds, and the number of molds was higher than the number of yeasts. Larger numbers of microorganisms were found from the air outside the churn than from the air inside the churn.

A striking decrease in the number of bacteria from the air was observed when a study was made comparing a churn covered with a muslin door and one unprotected.

Olson and Hammer (54) in another experiment studied the contamination of air by bacteria, yeasts, and molds by taking samples from four inside and two outside locations of a dairy industry building. In this experiment the authors used ninety-millimeter plates which were exposed for one hour to air and then incubated at 21° C. for four days. These workers concluded that the bacteria, yeasts, and molds were rather constantly found in air and in general, bacteria were in greater numbers than the yeasts. There was no great difference between the numbers of microorganisms found from the indoor and outdoor, and there was no distinct seasonal variation. A muslin cover over the plates reduced the number of organisms trapped from the air. The authors stated that the contamination of dairy products and plant equipment from the air could be expected and suggested that the consideration should be given for the air coming from outside in any attempt to control the air contamination in a plant.

Albert et al. (2) isolated Bacterium linens (now known as Brevibacterium linens) from the air of a dairy plant by exposing plates of Trypticase Glucose Extract Agar and incubating the plates at 8, 10, and 21° C. for one week. These authors mentioned that the sources for Bacterium linens as materials around stables, especially feeds, hay, straw, water, manure, and in the mouths of cows. This organism was described as gram-positive and rod shaped. Perry et al. (61) collected the air samples with a slit sampler from cheese making rooms of seven plants located in three different parts of England. These research workers incubated the plates at 30° C. for five days and found that the species of Lactobacillus genus namely, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, and Lactobacillus brevis were present in air.

Labots et al. (43) tried to estimate the airborne contamination of milk during bottling operations. In order to facilitate counting of plates high bacterial counts were achieved by nebulization of Streptococcus lactis culture with an indirect type of atomizer. The resulting aerosols were distributed in the experimental room using a fan. The bacterial count of the surrounding air during bottling

operations was found to vary for the several experiments from twenty thousands to sixty thousands per liter of air. The bacterial content of the empty bottles was two to three times higher than the corresponding volume of air. This was explained as being due to the probable sedimentation of bacteria into the bottles. These research workers concluded that a good correlation existed between the numbers of bacteria found in the milk and the numbers expected when bacteria in the empty bottles and in the air volumes flowing through the vent tubes and junction between valve and reservoir were trapped in the milk. These authors further stated that the actual contamination of bacteria through the air varied with the bacterial content of the air. However, these workers were uncertain about the relative role of airborne contamination in respect to the keeping quality of bottled milk when compared to the relative importance of contamination from other sources.

Balaprakasam (8) determined the microbiological content of the air in two dairy plants by using the Andersen air sampler (4). In this work Tryptone Glucose Extract Agar plates were incubated at 23 to 24^{o} C. for three days. Higher number of microorganisms was noticed in the air of

the first plant than was true for the second plant. This was explained because the first plant was located in a busier area of the city, with automobile traffic on either side of the plant, whereas the second plant was located in quieter surroundings. The air movement in the second plant was less even though the ventilation was good. The author attributed that the wet floors with occasional spillage together with movement of air due to electric fans and movement of personnel were contributory factors for higher number of microorganisms in the air of the first plant. The bacterial contents of the air from the bottle washing room and cottage cheese filling room of the first plant were significantly higher than the counts from the other rooms. The bacterial content near the bottle filler was four times higher than the bacterial content of the other locations in the same room of the first plant. No explanation could be offered as the conditions in the two parts of the room were similar.

The bacterial content of the air of the second plant did not exceed ten per cubic foot of air. Spacious accommodation, absence of undue air movements and the presence of only two workers in the second plant were attributed to be

the probable causes for the lower microbial content of the air of the second plant.

Balaprakasam (8) concluded that the outside weather seemed to have had no influence on the microbial counts of air inside the buildings. In coming to rather a contrary conclusion to hitherto accepted views the author felt that the only explanation possible might have been that the differing weather conditions outside the buildings may not have influenced the microbial contents of air within, so long as the inside was not completely exposed to the outside. The author's tentative classification of micro-organisms placed them in the genera Achromobacter, Alcaligenes, Brevibacterium, Bacillus, Corynbacterium, Flavobacterium, Microbacterium, Micrococcus, Pseudomonas, Streptococcus, and Stretomyces. Except Streptococcus and Streptomyces all other microorganisms exhibited visible growth at 5^{o} C. in five days.

In brief summary, a general conclusion could be made that the psychrophilic bacteria do not survive proper pasteurization. Further, the various reports on post-pasteurization contaminating sources directly or indirectly focus attention on the importance of systematic study of

such post-pasteurization sources of contamination in dairy plants.

VIII. KEEPING QUALITY

Keeping quality of milk could be affected by the presence of large numbers of non-pathogenic bacteria in milk. The consumer desires to have a fresh and good quality milk. Nicholas and Anderson (51) considered the freshness in the following terms:

> It is primarily a question of age measured by determination of flavor, decomposition of constituents, or increase in bacterial population. In general, spoilage or decomposition of perishables is the result of growth of microorganisms, but may be due to enzymatic reactions not dependent on bacterial multiplication. . . . Therefore until alterations can be noted in appearance, recognized in taste, or detected by laboratory tests, no change has occurred and the product may still be regarded as fresh.

These authors studied the keeping quality of pasteurized milk, homogenized-pasteurized milk, and raw milk under different conditions at 40° F. in a home refrigerator. Standard Plate Count and titratable acidity were conducted from the first day to the date of spoilage. The pasteurized or homogenized-pasteurized milk, with an initial bacterial count averaging 1 to 94 thousand per milliliter were found

to store for an average period of 17.2 days at 40° F.; whereas two separate batches of raw milk were not spoiled for four and seven days. Differences in types of initial contaminating bacteria were responsible for the variation in keeping time at 40° F. These research workers concluded that the low temperature organisms when present in raw milk in high proportions, spoiled the raw milk in a relatively short time, whereas the pasteurized milk even with large bacterial contents, like 93,000 per milliliter, had very good keeping quality. Further these authors concluded that the pasteurized or homogenized-pasteurized milk maintained good quality even after storage for twenty-eight days in a home refrigerator and was comparable to the quality of fresh milk by tests based on appearance, taste, odor, milk fat, total solids, and acidity. However, these authors stated that the pasteurized or homogenized-pasteurized milk may be stored in a home refrigerator at 40° F. for two weeks or possibly longer before spoilage can occur.

Conn (15) in 1903 investigated the relationship of temperature to the keeping quality of raw milk and stated that the storage temperature influenced the rate of bacterial multiplication; and further noted that the bacteria

multiplied only 5 times at 50° F. in twenty-four hours, while at 70° F. multiplication was 750 times during the same period. Conn (15) further stated that the raw milk preserved at 50° F. or lower, kept sweet for a longer time but contained more unwholesome types of bacteria; hence, old milk was unfit for marketing in spite of its sweetness. Dahlberg (17) made an extensive study in the New York metropolitan area during the cool fall month of October and the severe winter month of February and stated that the refrigerators in the home should not be above 50° F. to best safeguard the healthfulness of pasteurized milk. The same author observed that the growth of coliform bacteria at 45 to 50° F. was slow but significant.

Dahlberg (18) in another study, held freshly pasteurized milk at room temperature for six hours before refrigerating at 35 to 40° F. to simulate the failure of prompt refrigeration of delivered milk and found very slight increase in total bacterial count and coliform count in some samples. The Standard Plate Count was used in this study. The flavor scores of the milk held at room temperature for six hours before refrigeration were good for seven days. From this study, a conclusion was made by Dahlberg that the

keeping quality of pasteurized milk was good enough at all seasons of the year to permit every other day delivery of milk.

Burgwald and Josephson (14) observed that the initial Standard Plate Count and psychrophilic count did not indicate the potential keeping quality of pasteurized milk stored at about 40° F. Further, these research workers stated that the good quality milk retained excellent bacteriological and flavor qualities at least for four days during summer and for six to seven days during winter when the storage temperatures were maintained near 40° F. Boyd et al. (10) stated that the Standard Plate Count and either coliform or psychrophilic counts of freshly pasteurized milk did not help to predict the keeping quality of milk held at 33° F. or at 40° F. However, a correlation between the growth of psychrophilic bacteria and flavor deterioration was detected. In this work the keeping quality of commercially pasteurized and homogenized milk stored at 40° F. was investigated. The findings were that the milk kept well for thirteen to eighteen days based on a flavor score of 37 or above and a period of eight to eleven days with less than 50,000 organisms per milliliter. The duplicate samples

of the same milk stored for an additional period of eleven to fourteen days, when kept at 33° F. In summer, milk maintained superior quality when compared to winter samples.

Olson et al. (56) studied the growth of psychrophilic bacteria and their influence on the keeping quality of milk. This study revealed that the flavor defects in pasteurized milk kept at refrigeration temperatures were due to the production of metabolic products by bacteria. The type of species of bacteria and their numbers influenced the time required to produce the off flavors. These authors concluded that the Standard Plate Count was worthless in predicting the keeping quality, while the presence of coliforms even in small numbers in the fresh product was associated with poor keeping quality. However, these authors point out that the negative coliform samples could not be relied upon because the flavor deterioration could result from the presence of psychrophiles and hence milk could not be stored for long. In the same work, the authors stated that the psychrophilic bacterial count should be extremely low in large samples in order to assure good keeping quality of pasteurized milk; while the addition of antibiotics in small quantities had no effect in extending

the keeping quality of pasteurized milk. Lawton and Nelson (44) stated that the milk containing as low as one thousand psychrophilic organisms per milliliter increased to ten million per milliliter in 3 to 4 days even when stored at 5^{o} C.

Ford and Babel (26) studied the milk quality problems associated with present-day marketing. The authors' extensive trials indicated that the post-pasteurization contamination had much influence on the keeping quality of milk. In this study the milk containers were stored at 41 to 45^{o} F. throughout the distribution. These research workers added that the flavor scores decreased gradually when the milk was stored at 40 or at 45^{o} F.; all the samples had flavor criticisms after holding at 45^{o} F. for five days. These workers further observed that the psychrophilic bacteria were present in the milk tested from pasteurizer; and coliform contamination was more evident than the psychrophilic contamination in freshly bottled milk.

Marth and Frazier (46) stored raw milk at 36, 38, 45^{o} F. for four days and conducted Standard Plate Count, thermoduric and psychrophilic counts every day. On the average, at 36^{o} F. there was a decrease in the numbers of

bacteria during the first two days the psychrophiles increased at the end of three days and both Standard Plate Count and psychrophilic count increased after four days. A storage temperature of 36° F. was found to be satisfactory for holding raw milk for three days. These research workers observed that the psychrophilic bacteria grew more in raw milk samples held at 38 and 45° F. than those held at other temperatures and they grew at a greater rate in summer than in winter. Marth and Frazier (47) in another study indicated that very good raw milk could be held for three or four days at 36° F. without significant increase in numbers of bacteria. Further, these workers stated that storage temperature of raw milk at 36° F. was best for two days, and 38° F. was fairly good, while 45° F. was unsatisfactory.

The impossibility of predicting the keeping quality of milk by considering the initial psychrophilic count was further substantiated by Broitman et al. (12). These workers added that milk samples with less than ten organisms per milliliter in initial psychrophilic counts stored well for a longer time in refrigerators than did other samples. The other samples had ten to ten thousand psychrophilic organisms per

milliliter, and these samples, in turn, stored longer than the samples with ten thousand to one hundred million psychrophilic organisms per milliliter. The authors further stated that it would be very difficult to predict the shelf life based on psychrophilic counts. According to these research workers a positive test ("Nacconol-tri-phenyl tetrazolium chloride") in samples in twenty-four hours or less had poor keeping quality with nine days shelf life at 4.5° C. A positive test in twelve hours showed very poor keeping quality with a shelf life of four days at 4.5° C. A positive test in thirty-six hours suggests a shelf life of twelve days at 4.5° C., while a positive test in forty-eight hours indicates a shelf life of fifteen days. These authors concluded that a negative test at twenty-four hours indicates a good keeping quality of milk.

IX. DEFECTS

Off flavors and colors. The extensive contamination in raw or pasteurized milk with psychrophilic bacteria has resulted in the development of various off flavors and colors. Some of the off flavors commonly attributed to the activity of psychrophilic bacteria are unclean, putrid,

bitter, fruity, fishy, and unclean sour flavor (52, 55, 56, 72). Pereira and Morgan (59) noticed the fruity aroma production by all the six strains of Pseudomonas fragi which were studied. In another study, Pereira and Morgan (60) stated that fruity aroma defect caused by Pseudomonas fragi appeared to be due to the formation of a group of simple esters.

Seleen and Stark (67) isolated 169 organisms producing a green-fluorescent pigment from milk, butter, lactose, milk bottles, water, ground meat, fish, rabbit sera, bone meal, lettuce, wood pulp, soil, manure, and sewage. In addition to these isolates another thirty stock cultures were tested. Among these organisms 76 per cent grew at 5° C. but failed to grow at 42° C., whereas 21 per cent grew at 42° C. but not at 5° C. The production of green fluorescent-pigment was noticed to be a common character for all the cultures examined by the authors.

Georgia and Poe (30) stated that the presence of magnesium, phosphate, and sulfate were essential for fluorescent pigment production by bacteria in any medium. Foster et al. (27) described Pseudomonas cyanogenes as producing definite blue color in milk when some other acid-

producing organisms like Streptococcus lactis were present in milk. These authors stressed that abundant oxygen supply was necessary for the color production and cited the example of Bacterium linens (Brevibacterium linens) which had considerable decrease in the chromogenic activity in the absence of abundant oxygen supply. Hammer and Babel (32) stated that several organisms which were found in milk produced colored colonies, especially, yellow, orange, or red on various solid media. However, the authors mentioned that there was a difference in color production on solid media and in typical color fermentation in milk. Further, the authors reported on incidental isolations of yellow color-producing Pseudomonas synxantha from the milk that remained at room temperature until yellow color developed at the surface of the cream. These authors also mentioned the occasional presence of red color-producing organisms such as Serratia marcescens in milk.

Ingraham and Stokes (37) observed that the psychrophilic bacteria produce diffusable and non-diffusable pigments even at low temperatures. However, these research workers reported that the pigment production was not a common characteristic of psychrophilic bacteria though the

pigment production was an important factor in spoilage of fish, dairy, and other products. These authors further reported the correlation between rate of pigment production and rate of growth of psychrophilic bacteria. Schultze and Olson (65) stated that the majority of Pseudomonas species of 586 cultures produced diffusable fluorescent pigment when incubated on Long and Hammer agar at 7° C. for ten days. Parker et al. (58) stated that Pseudomonas fluorescens and Pseudomonas fragi produce rancidity; Pseudomonas viscosa causes dark color spoilage, associated with rotten or putrid odor, and produces greenish yellow pigment; Pseudomonas fragi causes light colored spoilage along with fruity odor; Alcaligenes metalcaligenes causes clear whitish spoilage.

Hiscox (33) isolated two strains of an organism from the butter samples which had developed blue-black discolorations when stored in a refrigerated chamber of a ship. This organism was described as gram-negative, nonsporulating rod with rounded ends, pleomorphic and actively motile with slow but abundant growth at 1 to 3° C. with pigment production. At 30° C. growth was satisfactory but no pigment was produced, while this organism did not grow at all at 37° C.

Authors considered that this organism belongs to the Pseudomonas genus but did not confirm the identification because of lack of any information of this nature in literature in 1936. Further this organism produced brownish color at 15° C. which diffused throughout the medium. However, at low temperatures this brownish color was mixed with blue-black pigment. A very dark color was produced when the growth of the organism was abundant at low temperatures.

Body defects. Davis and Babel (21) reported that seven out of nine psychrophilic cultures produced slime on cottage cheese held at 4.4° C. in seven days. Olson et al. (55) reported that the psychrophilic bacterial contamination of cottage cheese resulted in the formation of gelatinous slime or tapioca curd. In butter, contamination with psychrophilic organisms resulted in the defect, surface taint.

X. BIOCHEMICAL ACTIVITY

Lipolysis and proteolysis. Psychrophilic bacteria, besides producing off flavors and colors in milk and milk products, also bring about other deleterious effects which

are manifested in the form of lipolysis and proteolysis.

Lubert et al. (45) stated that the Pseudomonas fluorescens commonly occurred in raw milk and cream as a contaminate from the waters of the region and often produced rancidity in dairy products. Overcast and Skean (57) inoculated twenty-five pure cultures of lipolytic microorganisms which are sometimes found in regular market milk into high quality pasteurized milk and held at $4 \pm 1^{\circ}$ C. for twelve days. Seventeen cultures were responsible for rancid flavor together with or followed by bitterness while one culture caused the milk to be sour and unclean.

Witter (81) observed that the psychrophilic bacteria were generally strongly lipolytic, proteolytic, or both. The same author further stated that the holding of milk at low temperature could be a selective feature against acid producing bacteria and against psychrophilic bacteria.

Davis (20) stated that psychrophilic bacteria attack proteins and fats but do not attack lactose appreciably and hence clean milk held at low temperatures becomes stinking but does not become sour.

Peterson and Gunderson (62) studied the exo- and endocellular proteolytic enzyme systems of a culture of

Pseudomonas fluorescens isolated from frozen chicken pies. The culture grew and rapidly reproduced at 0, 5, 10° C. and showed marked sacchrolytic, lipolytic, and proteolytic activities. Extracellular enzyme production was more at an alkaline pH. The proteolytic activity increased as the temperature was raised from 0 to 20° C. but further raises in temperature decreased the proteolytic activity.

The majority of sixty-one coliforms isolated at 4° C. by Schultze and Olson (66) showed marked proteolytic tendencies and with less gas production from lactose than expected. These organisms caused alkaline proteolytic reaction in litmus milk. Jezeski and Macy (39) stated that the types of psychrophilic bacteria involved are more important than the numbers in causing defects in dairy products. However, these authors said that some of the inert psychrophiles may produce intense odor in cream without extensive proteolysis. Thomas and Druce (72) studied 235 psychrophilic cultures and 52 per cent of them caused proteolysis and the same study also revealed the fat-splitting property of the psychrophiles. Greene and Jezeski (31) stated the biochemical characters of some psychrophilic cultures used were not constant and varied as the incubation

temperature changed.

XI. CONTROL MEASURES

Since most available literature suggests that psychrophilic bacteria do not survive pasteurization, their presence in pasteurized milk or milk products could be attributed to post-pasteurization contamination. Hence, the emphasis on the control measures should be directed toward such contamination. Olson et al.(55) suggested the chlorination of water used to clean dairy equipment with five to ten parts per million of available chlorine to control psychrophiles, since most of them were found to be markedly sensitive to chlorine. The same authors further suggested proper pasteurization, proper cleaning and sanitizing of dairy equipment, protecting the equipment prior to use against recontamination and chlorination of cleaning water as the control measures against psychrophilic bacterial contamination.

Ingraham and Stokes (37) stated that food spoilage by psychrophilic bacteria can be reduced by observing proper sanitation during production, maintaining low temperatures, preferably below minus 10^{o} C. throughout distribution, and

reducing the time between production and consumption. Witter (81) stated that the hypochlorites are most effective bactericides to control psychrophiles and suggested a range of five parts per million for 5 seconds to fifty parts per million for 1 minute. Parker et al. (58) stated that the action of quaternary ammonium compounds or hypochlorites in eliminating psychrophilic bacteria in cleaning water or on equipment cannot be relied upon when applied to dirty surfaces. These research workers indicated that five to ten parts per million of available chlorine will eliminate all psychrophilic species unless they are hidden in a slime layer. Jezeski (38) suggested the treatment of cleaning water with hypochlorites at five to ten parts per million of available chlorine for 15 seconds and one hundred parts per million of available chlorine for 15 seconds for equipment sanitization.

CHAPTER III

EXPERIMENTAL METHODS

All the samples of air, water, milk, swab samples from dairy equipment, and carton stocks were collected from the University of Tennessee Creamery from April to August, 1965. The Creamery is located in a relatively clean and calm area free from traffic. Air samples were collected with an Andersen air sampler as recommended in Standard Methods for the Examination of Dairy Products (3). The air samples were collected within a distance of about one foot from the carton filling portion of the packing machine which is located in the pasteurization room. The swab samples were collected in accordance with standard procedures (3) from an eight square inch area of the equipment, namely the homogenizer, the heat exchanger, the pipeline, and the pasteurized milk-holding tank, after chlorination and a few minutes before the starting of pasteurization process. Beginning with the third trial, swab samples from eight square inch parts of carton stocks were also collected.

These swabs were immersed in about eight milliliters of buffer rinse solution, and this solution was distributed on Milk-Protein Hydrolysate agar plates. Water from the dairy plant and after the first trial, ten milliliter samples of pasteurized milk were plated on Milk-Protein Hydrolysate agar to determine the presence of psychrophiles.

I. DESCRIPTION OF THE AIR SAMPLER

The portable Andersen air sampler (4) operates on the principle of impingement of particles on a solid surface of the culture medium and classifies the airborne particles into six aerodynamic sizes. The sampler includes six successive stages through which the sample of air is drawn. Each stage consists of plates perforated with four hundred holes and immediately below that, provision is made to keep the special petri dish containing approximately twenty-seven milliliters of the media. The number of the holes is constant in all the six stages but the size of the individual holes decreases from top to bottom. This sampler is pressure sealed and thereby forces only the measured air through the perforated discs. This sampler is operated electrically and draws a measured one cubic foot of air per

minute. All the visible colonies on the medium were counted after the completion of incubation period using the Quebec colony counter. The positive hole method recommended by the manufacturers of this instrument was not used since the total number of colonies was generally small.

II. MEDIA USED

Milk-Protein Hydrolysate (M-PH) agar was used as the culture media for the enumeration of bacteria from all the collected samples.

III. INCUBATION TEMPERATURES

During the first five trials, all the samples were incubated at 21° C. for three days which resulted in isolation of some spore formers which were not psychrophiles. Thereafter all samples were incubated at 5° C. for fifteen days. The fifteen day incubation period at 5° C. rather than the seven to ten days at 5° to 7° C. recommended in standard procedures (3) was practiced since colony counts after fifteen day incubations were observed to be consistently higher than colony counts after ten days.

IV. ISOLATION AND PURIFICATION

After the scheduled incubation period, typical individual colonies were removed with an inoculation needle into litmus milk and then incubated for three days at 21° C. After incubation, a loopfull of litmus milk was transferred to M-PH agar slant and these were incubated at 21° C. for three days. Again the colonies were picked and inoculated into litmus milk and incubated for three days at 21° C. These litmus milk cultures were transferred with a loop to M-PH agar slants which were incubated for three days at 21° C. These were considered as pure cultures for this experiment and were stored in a cold storage room (at 4 to 7° C.) until further tests were conducted.

The following tests were conducted after reinoculation into trypticase soy broth and incubating for three days at 21° C. All the tests were conducted in accordance to Manual of Microbiological Methods (69) unless otherwise stated.

1. Gram Stain. While staining the unknown organisms, known organisms like Escherichia coli (gram negative) and Micrococcus conglomeratus (gram positive) were used as controls.

2. Colonial and cellular morphology test after three days incubation at 21° C. on Trypticase soy agar slants.
3. Motility test as suggested by Tittsler and Sandholzer (79).
4. Temperature relationships established according to growth in Trypticase soy broth at :
   a. 0° C. for about one month's time.
   b. 4 to 6° C. for about one month's time.
   c. 32° C. for seven days.
   d. 37° C. for three days.
5. Action on litmus milk tested after incubation at 21° C. for three to seven days.
6. Liquification of gelatin tested at 21° C. for seven days.
7. Nitrate reduction test.
8. Carbohydrate metabolism checked according to the method described by Hugh and Leifson (34). This test was employed to distinguish between fermentative and oxidative metabolism of carbohydrates by various organisms.

9. Production of water soluble pigments tested as described by Georgia and Poe (30) for fifteen days at 21° C.

10. Butterfat hydrolysis test conducted for ten days at 21° C. as described by Knaysi (42).

The cultures were classified on the basis of these tests in accordance to Skerman's Guide (68) which confirms classification given in Bergey's Manual (11).

CHAPTER IV

RESULTS AND DISCUSSION

An attempt was made to further explore the various sources of psychrophilic bacteria with particular emphasis on air as one of the possible sources. The air samples were collected in twelve trials, other samples were collected from equipment and water in eleven trials. Also, the samples from fresh pasteurized milk were collected in ten trials, samples from stored (at 5° C. for ten days) pasteurized milk were collected in six trials, and samples from carton stock were collected in nine trials.

The data in Table I show the numbers of psychrophilic bacteria counted from one cubic footsamples of air within one foot distances from the filler of the packing machine. The samples were collected on either side of the machine in all twelve trials. The psychrophilic counts varied from zero to thirty-six per cubic foot of air. The average number of psychrophilic bacteria present in one cubic foot of air for the twelve trials was fourteen on either side of

TABLE I

COUNTS OF PSYCHROPHILIC BACTERIA FROM ONE CUBIC FOOT OF AIR COLLECTED AT A DISTANCE OF ONE FOOT FROM THE FILLER

	Plates Incubated at 21° C. for Three Days					Plates Incubated at 5° C. for Fifteen Days							Average for Twelve Trials
	Trial					Trial							
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	
Right Side	23	20	8	0	21	12	23	4	6	32	12	9	14
Left Side	32	3	2	23	8	36	20	2	0	27	9	7	14
Average per Each Trial	28	12	5	12	15	24	22	3	3	30	11	8	

the machine. The same average counts on both sides of the machine, based on twelve trials, indicated that there was no difference in the average numbers of airborne psychrophilic bacteria on either side of the machine. This can be expected since the distance between either side of the machine where the sampler was operated was only one foot.

One cubic foot of air (approximately 28,317 milliliters) can fill about twenty-eight quart cartons. The average count of airborne psychrophiles per one cubic foot of air was fourteen. On this basis, an assumption could be made that one of the fourteen organisms could possibly gain entrance into one of two quart cartons or a single organism might enter a half-gallon carton. El-Farekh (24) found that the generation time for Pseudomonas fragi was 5.50 hours, for two cultures of Pseudomonas fluorescens was 7.22 hours, and for Brevibacterium lipolyticum was 11.24 hours. All these generation times were calculated after maintaining the cultures at 4 $\pm$ 1° C. in skim milk. Olson (52) calculated the theoretical shelf life of a quart of milk containing one vigorously growing organism and stored at 45° F. According to these calculations a single organism in a quart of milk and with a generation time of six hours

can multiply to 1 million per milliliter in eight days, or to 73 millions per milliliter in nine days. Similarly, a single organism per quart of milk and with a generation time of twelve hours can multiply to 1 million per milliliter in fifteen days and to 73 millions per milliliter in eighteen days. These references by El-Farekh (24), and Olson (52) emphasize the seriousness of even a single organism which could gain entrance into a milk carton through air or other means of contamination.

Only further work in this area can advocate psychrophilic bacterial standards in air. In controlling the psychrophilic bacterial contamination of milk and its products through air, consideration should be given to the outside air coming into the plant as suggested by Olson and Hammer (54). However, this study does not conclude that air could be a major source of contamination of psychrophilic bacteria in a dairy plant. Irrespective of the major or minor role of the air as a contaminating source, every precaution should be taken to avoid all contamination. This work confirms the views expressed by Labots et al. (43) that the actual contamination through air varies with the bacterial content of the air, though the relative role of

airborne contamination in respect to the keeping quality of milk when compared to the importance of contamination through other sources has not been clearly established.

The psychrophilic counts from eight square inch areas of dairy equipment, namely homogenizer, heat exchanger, pipeline, and pasteurized milk-holding tank, are tabulated in Table II. Swab samples from two different eight square inch areas of the same piece of equipment were plated in each trial except in trials four and five. Taking the combined average for all the twenty samples plated from two different eight square inch areas, the number of psychrophilic bacteria was more than thirty-six from homogenizer, six from heat exchanger, eight from pipeline, and more than thirty-four from pasteurized milk-holding tank. The relatively higher number of bacteria from the homogenizer could be explained because of difficulty in cleaning that part of the equipment. In the case of other parts, particularly the pasteurized milk-holding tank where the large parts were flat surfaces, the higher counts may be due to inadequate cleaning and sanitizing.

No standards for psychrophilic bacteria on equipment were suggested in Standard Methods (3). But Standard

TABLE II

COUNTS OF PSYCHROPHILIC BACTERIA FROM SWAB SAMPLES OF EIGHT SQUARE INCH AREAS OF DAIRY EQUIPMENT

Sample Source	Sample Number	Plates Incubated at 21° C. for Three Days, Trial #1	#2	#3	#4	#5	Plates Incubated at 5° C. for Fifteen Days, Trial #6	#7	#8	#9	#10	#11	Total	Average for Two Samples
Homogenizer	1	61	47	18	40	89	0	1	2	0	30	0	288	>36
	2	27	75	0	a	a	13	0	0	18	>300	0	433	
Heat Exchanger	1	0	2	0	2	13	8	0	12	12	6	0	55	6
	2	0	4	0	a	a	8	1	2	40	1	0	56	
Pipeline	1	1	7	1	24	35	5	0	0	0	20	0	93	8
	2	4	0	0	a	a	11	0	9	6	30	0	60	
Pasteurized Milk-Holding Tank	1	26	2	1	4	5	5	0	4	0	9	0	56	>34
	2	4	1	0	a	a	>300	>300	1	20	7	0	>633	

[a]Samples not plated.

Methods indicate that, when differential media have been employed to find the sources of post-pasteurization contamination, the results obtained should be negative since the coliforms, psychrophiles, yeasts, and molds do not generally survive pasteurization temperatures or proper sanitization procedures. In addition the available literature (38,55,81) indicates that the treatment of the equipment with five to ten parts per million of available chlorine for 5 to 15 seconds usually destroys all psychrophilic bacteria. The same explanation, offered primarily in the case of slight contamination from air and based on supporting evidence from two different authors (24, 52) would seem to be valid and fitting when applied to the small number of psychrophilic bacteria on the equipment. Hence, the dairy industry should emphasize thorough cleaning of all parts of the equipment and maintain frequent checks on the sanitary process to assure good keeping quality of the product.

The data in Table III present the counts of psychrophilic bacteria from swab samples made from eight square inch areas of carton stock, and from ten milliliter portions of water from the water supply of the dairy plant. The

TABLE III

COUNTS OF PSYCHROPHILIC BACTERIA FROM SWAB SAMPLES OF EIGHT SQUARE INCH AREAS OF CARTON STOCK AND FROM TEN MILLILITER SAMPLES OF WATER

	Plates Incubated at 21° C. for Three Days					Plates Incubated at 5° C. for Fifteen Days							
	Trial					Trial							
Sample Source	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	Total	Average
Swab Samples From Eight Square Inch Areas From Carton Stock	a	a	0	1	3	0	0	0	13	19	0	36	4
Ten Milliliter Samples of Water From the Dairy Plant	0	51	4	b	105	1	0	0	20	12	0	193	>19

[a] Samples not plated.

[b] Spreader was observed.

numbers of bacteria from eight square inch parts of the carton stock varied from zero to nineteen with an average of four counts per eight square inches. Although the counts from four samples were zero, the remaining five samples had counts ranging between one and nineteen. This suggests that the carton may act as a serious source of psychrophilic contamination. Further work in this area is needed if appropriate remedies or checks are to be proven and suggested.

The counts from ten milliliter portions of water varied from zero to 105 with an average of more than nineteen bacteria per ten milliliters. Standard Methods (3) states that psychrophilic bacteria do not survive chlorination of water and, hence, does not suggest any specific standard for psychrophilic bacteria in water. However, Standard Methods (3) does suggest that the count should not be more than five "proteolytic and/or lipolytic organisms" per milliliter of water. Since most of the psychrophilic bacteria and particularly the psychrophiles identified in this study were both proteolytic and lipolytic, indications are that water may play a part in contamination with psychrophilic bacteria. This is further

emphasized by the fact that potable water supplies may not be satisfactory for washing cottage cheese--the general practice being to add four to six parts per million of available chlorine just prior to washing the cheese.

The number of psychrophilic bacteria from ten milliliters of fresh pasteurized milk, and from ten milliliters of stored (at 5° C. for ten days) pasteurized milk are tabulated in Table IV. These samples were plated just to learn the psychrophilic counts in the respective samples and to identify the organisms. They do not necessarily reflect the sources of contamination. Ten milliliter samples of fresh pasteurized milk were plated in 10 trials and the count varied from zero to twenty-four, with an average of more than three per ten milliliters. Here again, no standards were suggested by Standard Methods (3), although a caution was noted indicating that low counts do not imply the inability of the bacteria to grow and multiply to undesirable numbers during normal refrigeration storage. In three instances (trials seven, nine, and eleven) the counts in the stored milk were appreciably higher than in the fresh milk. This situation was particularly noticeable in trial nine, where a zero count was recorded for the ten

TABLE IV

COUNTS OF PSYCHROPHILIC BACTERIA FROM TEN MILLILITER SAMPLES OF FRESH AND STORED PASTEURIZED MILK

	Plates Incubated at 21° C. for Three Days					Plates Incubated at 21° C. for Fifteen Days							
	Trial					Trial							
Sample Source	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	Total	Average
Ten Milliliter Samples of Fresh Pasteurized Milk	a	2	b	24	4	0	1	0	0	0	0	31	>3
Ten Milliliter Samples of Stored Pasteurized Milk	a	a	a	a	a	2	>100	0	>300[c]	d	12	>414	>82

[a] Samples not plated.

[b] Spreader was observed.

[c] These colonies were golden yellow in color.

[d] Milk curdled and developed a rancid smell on storage at 5° C. for ten days and hence was not plated.

milliliters of fresh pasteurized milk, while the corresponding count for stored pasteurized milk was more than three hundred per ten milliliters. Such differences in the fresh and stored milk could definitely be attributed to the growth of psychrophilic bacteria during refrigerated storage.

In trial ten, though the number of bacteria was zero in fresh milk, on storage for ten days at 5^{o} C., the milk was curdled and developed a rancid smell and hence the stored milk was not plated. This kind of situation is an interesting one from an inferential point of view, and can be explained by the fact that a mere absence of psychrophilic bacteria in a given ten milliliter sample does not indicate that the milk is free from psychrophilic bacteria. The counts in stored pasteurized milk varied from zero to more than three hundred per ten milliliters of stored milk with an average count of more than eighty-two per ten milliliters.

The detailed particulars of the different tests conducted to identify the organisms were tabulated in Appendixes A, B, C, and D. The following tests were conducted:

1. Gram stain.
2. Morphology.
3. Motility.
4. Growth at 0, 4 to 6, 32, 37° C.
5. Action on litmus milk.
6. Liquefaction of gelatin.
7. Nitrate reduction.
8. Carbohydrate metabolism.
9. Pigment production.
10. Butterfat hydrolysis.

Some of the tests have a direct bearing on the operations of the dairy industry and are briefly discussed in the following pages.

The organisms identified in this work belonged to the genera Pseudomonas, Brevibacterium, and Arthrobacter. The majority of the identified organisms (94 out of 136) belonged to the genus Pseudomonas, this being in conformity with the views expressed by a number of research workers (13, 16, 29, 39, 48, 55, 64, 65, 72, 81). Twenty-seven out of 136 identified organisms were found to belong to genus Brevibacterium, while another fifteen were identified to be motile species of genus Arthrobacter. Some of the organisms

of these two genera, namely Brevibacterium and Arthrobacter, also were reported by previous workers (2, 6, 8, 72, 81), as psychrophilic bacteria.

In all probability, most of the organisms identified from the air, and all the organisms identified from various parts of the equipment, carton stock, and water could have gained entrance into pasteurized milk. These organisms were capable of bringing about the many reactions in milk which eventually render it unfit for consumption.

The data in Table V show the characteristics of these identified organisms that may be of practical importance to the dairy industry. All of the 136 organisms identified from various sources grew at 4 to 6° C., while 129 of them grew at 0° C. This would indicate that when these organisms gain entrance into milk, they could grow and bring about undesirable changes even at relatively low temperatures. Of 136 identified organisms, only one was inert and did not cause any change in litmus milk, while the large majority of them (94 out of 136) caused alkaline reaction together with or without reduction of the litmus milk. The alkaline reaction in litmus milk may be interpreted as proteolytic action (66) which would mean the milk with such proteolytic

TABLE V

DETAILS OF THE TESTS SHOWING RELATIONSHIPS BETWEEN THE IDENTIFIED PSYCHROPHILIC BACTERIA AND OPERATIONS OF THE DAIRY INDUSTRY

Growth at		Green Fluorescent Pigment Production	Action on Litmus Milk*						Butterfat Hydrolysis		
					Alk & Red	Acid & Red	Acid Red & Co	Only Red		Weak Hydrolysis	Complete Hydrolysis
0°C	4-to-6°C		N A	Alk					N A		
129	136	19	1	28	66	16	2	23	29	12	95

*KEY:
- N A - No Action
- Alk - Alkaline
- Alk & Red - Alkaline and Reduction
- Acid & Red - Acid and Reduction
- Acid Red & Co - Acid, Reduction, and Coagulation
- Only Red - Only Reduction

changes would be undesirable for human consumption. Sixteen organisms reacted in litmus milk by producing acid and reduction, while two organisms produced acid, reduction, and coagulation in the milk. This production of acid would make the milk sour and unfit for the consumption.

A large majority of the identified orgaisms (95 out of 136) produced complete hydrolysis in butterfat, while another twelve organisms caused weak hydrolysis. The remaining twenty-nine organisms did not cause any reaction. The large majority of these identified organisms when present in milk would bring about the lipolytic changes in milk fat which have been associated with stale and rancid flavors.

In view of the facts and explanations given, a statement that these identified organisms when present in milk would bring about deleterious effects would be appropriate.

The identified organisms are shown, with their sources of isolation, in Table VI. However, this table does not represent the types of organisms in the mutual relation of one source of isolation to another. This was because a comparison between one cubic foot of air, eight square inch portions of equipment and cartons,

TABLE VI

CHARACTERIZATION OF IDENTIFIED PSYCHROPHILIC BACTERIA FROM DIFFERENT SOURCES

Source	Fluorescent Species of *Pseudomonas* Genus	Non-Fluorescent Species of *Pseudomonas* Genus		Species of *Brevibacterium* Genus	Species of *Arthrobacter* Genus	Total Number of Isolates[a]
		Motile Species	Non Motile			
Air	5	43	2	17	10	77
Dairy Equipment	11	11	4	5	5	36
Carton Stock	-	2	-	-	-	2
Water	1	2	-	1	-	4
Fresh Pasteurized Milk	-	6	-	4	-	10
Stored Pasteurized Milk	2	3	2	-	-	7

[a]No relationship exists among the total number of isolates from different sources.

and ten milliliter portions of water, fresh and stored pasteurized milk would be unjustifiable. Emphasis was placed on air as a possible source of contamination, and the results from the various sources were represented in a single table because of convenience.

Fifty out of seventy-seven organisms isolated and identified from air belonged to genus Pseudomonas, seventeen were species of Brevibacterium, while the rest were species of Arthrobacter. Out of thirty-six organisms identified from parts of the dairy equipment, twenty-six were species of genus Pseudomonas, five were species of Brevibacterium, and five were species of Arthrobacter. The high proportion of organisms of the Pseudomonas genus identified from air, equipment, and other sources may be explained because their natural habitat is usually water. These organisms find their way into the air through the splashing of water on the floors of the milk plant and dirt and dust particles present in the surroundings.

Of four organisms identified from water, three were Pseudomonas species while the remaining one was a Brevibacterium species. The two organisms identified from swab samples of carton stock, and the seven organisms identified

from stored pasteurized milk were Pseudomonas species. Six out of ten identified organisms from fresh pasteurized milk were Pseudomonas species, while the remaining four were Brevibacterium species.

None of the organisms identified from swab samples of carton stock, water, fresh and pasteurized milk were species of Arthrobacter genus. This may be due to the fact that the natural habitat for this genus seems to be soil. The normal precautions that would be taken in a dairy plant would appear to minimize soil-related contamination.

CHAPTER V

SUMMARY AND CONCLUSIONS

Some of the possible sources of psychrophilic bacterial contamination in a dairy plant were studied with an additional emphasis on air as a possible source. Air samples collected with an Andersen air sampler, swab samples from eight square inch portions of homogenizer, heat exchanger, pipeline, pasteurized milk holding tank, and carton stock were plated to determine the psychrophilic bacterial count. In addition to this, water as a source, and fresh and stored pasteurized milk also were plated.

A total of 136 organisms was identified to genus level based on Skerman's Guide (68) and in conformity with Bergy's Manual (11). The isolated organisms belonged to the genera Pseudomonas, Brevibacterium, and Arthrobacter. Among the ninety-four identified organisms of Pseudomonas genus, nineteen produced green fluorescent pigment.

From this work the conclusion that air is a major source of psychrophilic bacteria cannot be made. However,

indications are that air contamination could be responsible for the presence of a very small number of psychrophilic bacteria which are most undesirable in milk. The average number of airborne psychrophilic bacteria was fourteen per cubic foot of air.

Since most of the identified organisms showed evidence of deleterious effects in the tests employed which had a direct relation on operations of the dairy industry, every possible precaution in avoiding the contamination of psychrophilic bacteria from any source irrespective of the major or minor role of the sources was suggested.

Further work must be conducted in this area before psychrophilic bacterial standards may be recommended for air, dairy equipment, carton stock, water, and, finally, for fresh pasteurized milk.

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APPENDIXES

APPENDIX A

GREEN FLUORESCENCE PRODUCING GRAM-NEGATIVE SHORT RODS

(*PSEUDOMONAS* SPECIES)

TABLE VII

GREEN FLUORESCENCE PRODUCING GRAM-NEGATIVE SHORT RODS
(PSEUDOMONAS SPECIES)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Fer	Met Oxi	Butterfat Hydrolysis
31	Incubated at 5°C for fifteen days	+	--Green Fluorescence was Produced--	+	+	+	-	a	+	-	-	++	+
33		+		+	+	+	-	a	-	-	-	+	+
35		+		+	+	+	+	a	-	-	-	-	+
60		+		+	+	+	+	b	+	+	-	±	+
61		+		+	+	+	+	b	+	+	++	++	+
137	21°C 3 days	+		+	+	+	+	b	-	-	±	++	+
158	Incubated at 5°C for fifteen days	+		+	+	+	+	a	+	-	-	±	+
159		+		+	+	+	+	a	+	-	-+-	±	+
160		+		+	+	+	+	a	-	-	-+-	±	+
161		+		+	+	+	+	a	+	-	-	±	+

TABLE VII (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Met Fer	Cab Met Oxi	Butterfat Hydrolysis
164	Incubated at 5° C for fifteen days	+	Green fluorescent pigment was produced	+	+	+	+	a	+	-	-	±	+
165		+		+	+	+	+	a	+	-	-	±	+
166		+		+	+	+	+	a	+	-	-	±	+
167		+		+	+	+	+	a	+	-	-	±	+
168		+		+	+	+	+	a	+	-	-	±	+
169		±		+	+	+	+	a	+	-	-	±	+
170		+		+	+	+	+	a	+	-	-	±	+

TABLE VII (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Met Fer	Cab Met Oxi	Butterfat Hydrolysis
179	Incubated at 5° C for fifteen days	+	Green fluorescent pigment was produced	+	+	+	+	a	-	+	-	±	+
180		+		+	+	+	+	a	-	+	-	±	+

*KEY (Column captions):
C# - Culture number
O IN T - Original incubation temperature
Mot - Motility
Pig Prod - Pigment production
A L M - Action on litmus milk
Liq Gel - Liquefaction of gelatin
Red Nit = Reduction of nitrates
Cab Met - Carbohydrate metabolism
Fer - Fermentation
Oxi - Oxidation

KEY (Table Interior):
a - Alkaline reaction
b - Alkaline and reduction
+ - Positive
- - Negative
± - Weak

APPENDIX B

GRAM-NEGATIVE SHORT RODS

(*PSEUDOMONAS* SPECIES)

TABLE VIII

GRAM-NEGATIVE SHORT RODS
(PSEUDOMONAS SPECIES)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Fer	Met Oxi	Butterfat Hydrolysis
1	Incubated at 21° C for three days	+	-	+	+	+	+	a	-	-	±	a	+
4		+	-	+	+	+	+	e	-	-	±	±	±
6		+	-	+	+	+	+	e	-	-	-	-	±
7		+	-	+	+	+	+	e	-	-	-	-	+
8		+	-	+	+	+	+	e	-	-	±	±	-
14		+	-	+	+	+	+	e	-	-	++	++	+
17		+	-	+	+	+	+	a	-	-	-	+	++
19		+	-	+	+	+	+	b	+	+	++	++	-
20		+	-	+	+	+	+	a	-	+	++	++	-
21		+	-	+	+	+	+	c	-	-	±	±	±

TABLE VIII (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Met Fer	Cab Met Oxi	Butterfat Hydrolysis
26	Incubated at 5° C for fifteen days	+	-	+	+	+	+	e	-	-	±	±	+
29		+	-	+	+	+	-	b	-	-	±	±	-
30		+	-	+	+	+	-	b	-	-	±	±	-
32		+	-	+	+	+	-	b	-	-	±	a	+
37		+	-	+	+	+	-	e	-	-	+	+	+
41		+	-	+	+	+	+	b	-	-	±	±	±
42		+	-	+	+	+	+	e	-	+	±	a	+
45		+	-	+	+	+	+	b	-	-	-	±	+
46		+	-	+	+	+	+	b	-	-	-	-	+
57		+	-	+	+	+	+	b	-	-	++	++	+
63		+	-	+	+	+	+	c	-	-	++	++	++

TABLE VIII (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Fer	Met Oxi	Butterfat Hydrolysis
65	Incubated at 5° C for fifteen days	+	-	+	+	+	+	c	-	-	++	++	+
67		+	-	+	+	-	-	b	+	+	±	±	+
69		+	-	+	+	+	-	b	+	-	++	++	+
70		+	-	+	+	+	+	e	-	-	±	±	+
74		+	-	+	+	+	+	b	-	+	±	±	++
75		+	-	+	+	+	+	b	-	+	±	±	+
78		+	-	+	+	+	+	b	-	-	+	+	+
80		+	-	-	+	+	+	b	-	+	-	a	+
83		+	-	+	+	+	-	b	-	-	-	-	+
86		+	-	+	+	+	+	b	+	+	±	a	+
87		+	-	+	+	+	+	b	-	-	-	±	+

TABLE VIII (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Met Fer	Cab Met Oxi	Butterfat Hydrolysis
88	Incubated at 5° C for fifteen days	+	-	+	+	+	+	b	-	-	+	+	+
89		+	-	+	+	+	+	b	-	-	-	+	+
90		+	-	+	+	+	+	b	-	-	-	-	+
91		+	-	+	+	+	+	b	-	+	-	a	+
92		+	-	+	+	+	+	b	-	+	-	-	+
94		+	-	+	+	+	+	b	-	-	±	±	+
98		+	-	+	+	+	+	c	+	+	±	±	+
99		+	-	+	+	+	+	b	+	-	-	±	±
100		+	-	+	+	+	+	b	-	+	±	++	+
101		+	-	+	+	+	+	b	-	-	-	a	+
108		+	-	+	+	+	+	b	+	-	++	++	+
125	21° C 3 days	+	-	-	+	+	+	b	-	+	±	-	+

TABLE VIII (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Fer	Met Oxi	Butterfat Hydrolysis
126		+	-	+	+	+	+	b	-	-	-	a	-
127		+	-	+	+	+	+	b	-	+ -	±	+	-
131		+	-	+	+	+	+	b	+	+	+ -	a	+
134		+	-	+	+	+	-	None	+	+	±	±	+
135		+	-	-	+	+	+	b	+	+	±	±	+
138		+	-	+	+	+	+	b	-	-	+ -	a	+
139		+	-	+	+	+	+	b	-	-	±	a	+
145		+	-	+	+	+	+	b	+	+	+ -	+ -	+
147		+	-	+	+	+	+	b	+	+	±	-	+
149		+	-	+	+	+	+	b	+	+	±	-	+
175	5° C Incubated at 21° C for three days 15 days	+	-	+	+	+	+	b	+	-	±	+	+

TABLE VIII (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Met Fer	Cab Met Oxi	Butterfat Hydrolysis
176	Incubated at 5° C for fifteen days	+	-	+	+	+	+	a	+	-	±	a	-
178		+	-	+	+	+	+	a	-	-	±	±	+
182		+	-	+	+	+	-	e	-	+	±	+	-
190		+	-	+	+	+	+	e	-	+	±	±	+
196		+	-	+	+	+	+	e	-	-	±	±	+
201		+	-	+	+	+	+	a	-	-	±	a	-
202		+	-	+	+	+	+	a	-	-	±	a	-
205		+	-	+	+	+	-	e	-	-	±	±	-
215		+	-	+	+	+	+	c	-	-	±	+	+
223		+	-	+	+	+	-	b	-	-	±	-	+
225		+	-	+	+	+	-	b	-	-	-	-	+

TABLE VIII (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Met Fer	Cab Met Oxi	Butterfat Hydrolysis
226	Incubated at 5° C for fifteen days	+	-	+	+	+	+	a	+	+	++	++	+
55		-	-	+	+	+	+	b	-	-	-	+/-	-
81		-	-	+	+	+	+	b	-	-	-	a	+
177		-	-	+	+	+	+	b	-	+	++	++	-
197		-	-	+	+	+	+	b	-	-	+/-	+/-	-
203		-	-	+	+	+	+	a	-	-	+/-	a	-
210		-	-	+/-	+	-	+/-	b	-	-	+/-	+/-	-
214		-	-	+	+	+	+	c	-	-	-	+	+

TABLE VIII (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Met Fer	Cab Met Oxi	Butterfat Hydrolysis
227	5° C 15 days	-	-	+	+	+	+	c	+	+	++	++	+

*KEY (Column captions):
C# - Culture number
O IN T - Original incubation temperature
Mot - Motility
Pig Prod - Pigment production
A L M - Action on litmus milk
Liq Gel - Liquefaction of gelatin
Red Nit - Reduction of nitrates
Cab Met - Carbohydrate metabolism
Fer - Fermentation
Oxi - Oxidation

KEY (Table interior):
a - Alkaline reaction
b - Alkaline and reduction
c - Acid and reduction
e - Only reduction
+ - Positive
- - Negative
± - Weak

APPENDIX C

GRAM-POSITIVE RODS (*BREVIBACTERIUM* SPECIES)

TABLE IX

GRAM-POSITIVE RODS (BREVIBACTERIUM SPECIES)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-5° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Met Fer	Cab Met Oxi	Butterfat Hydrolysis
2	Incubated at 21° C for three days	+	-	+	+	+	+	c	-	-	±	±	+
3		+	-	+	+	+	+	a	-	-	±	a	+
5		+	-	+	+	+	+	e	-	-	±	-	±
22		+	-	+	+	+	+	c	-	-	±	++	±
23		+	-	+	+	+	+	e	-	-	±	±	±
24		+	-	+	+	+	+	e	-	-	±	±	±
25	Incubated at 5° C for fifteen days	+	-	+	+	+	+	b	-	+	++	±	+
27		+	-	+	+	+	-	e	-	-	+	+	-
28		+	-	+	+	+	+	b	-	-	±	±	-

TABLE IX (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Met Fer	Cab Met Oxi	Butterfat Hydrolysis
50	Incubated at 5° C for fifteen days	++	-	+	+	+	+	c	+	-	++	++	+
85		+	-	+	+	+	+	b	-	+	+-	+-	+
102		+	-	+	+	+	+	c	+	-	+-	+-	+-
103		+	-	+	+	+	+	b	+	-	+-	+-	-
104		+	-	+	+	+	+	d	+	-	++	++	-
105		+	-	-	+	+	+	b	-	-	+-	+-	-
106		+	-	-	+	+	+	b	-	-	+-	++	-
107		+	-	+	+	+	+	b	-	-	++	+	+

TABLE IX (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Fer	Met Oxi	Butterfat Hydrolysis
124	Incubated at 21° C for three days	+	-	+	+	+	+	b	+	+	±	+	+
128		+	-	+	+	+	+	b	-	±	±	+	+
129		+	-	+	+	+	+	b	+	-	++	++	+
130		+	-	-	+	+	+	b	+	+	±	±	+
144		+	-	+	+	+	+	e	-	-	-	a	+
148		+	-	+	+	+	+	b	+	+	±	-	+
155		+	+	+	+	+	+	a	-	+	±	-	-
157		+	-	+	+	+	+	b	+	+	±	a	+

TABLE IX (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Met Fer	Cab Met Oxi	Butterfat Hydrolysis
218	5° C 15 days	+	-	+	+	+	+	c	±	+	±	±	+
222	5° C 15 days	+	-	+	+	+	-	b	-	-	-	-	-

*KEY (Column captions):
C# - Culture number
O IN T - Original incubation temperature
Mot - Motility
Pig Prod - Pigment Production
A L M - Action on litmus milk
Liq Gel - Liquefaction of gelatin
Red Nit - Reduction of nitrates
Cab Met - Carbohydrate metabolism
Fer - Fermentation
Oxi - Oxidation

KEY (Table interior):
a - Alkaline reaction
b - Alkaline and reduction
c - Acid and reduction
d - Acid, reduction, and coagulation
e - Only reduction
+ - Positive
- - Negative
± - Weak

APPENDIX D

GRAM-VARIABLE RODS AND COCCI

(*ARTHROBACTER* SPECIES)

TABLE X

GRAM-VARIABLE RODS AND COCCI (ARTHROBACTER SPECIES)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Met Fer	Cab Met Oxi	Butterfat Hydrolysis
15	21° C 3 days	+	-	+	+	+	+	e	-	-	±	-	-
56	Incubated at 5° C for fifteen days	+	-	+	+	+	+	c	+	-	++	++	-
59		+	-	+	+	+	+	c	-	-	++	++	+
62		+	-	+	+	+	+	c	-	-	++	++	+
64		+	-	+	+	+	+	c	-	-	++	+	++
79		+	-	+	+	+	+	e	-	-	-	-	+
82		+	-	+	+	+	-	b	-	-	-	a	+
84		+	-	+	+	+	+	b	-	-	±	±	+
100		+	-	+	+	+	+	b	-	+	±	++	+

TABLE X (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at C	Growth at 4-6° C	Growth at 32° C	Growth at 37° C	A L M	Liq Gel	Red Nit	Cab Met Fer	Cab Met Oxi	Butterfat Hydrolysis
109	5° C 15 days	+	-	+	+	+	+	d	-	-	++	++	-
141		+	-	-	+	+	+	e	+	+	++	±	+
142	Incubated at 21° C for three days	+	-	+	+	+	+	b	+	+	±	++	+
146		+	-	+	+	+	+	b	+	+	±	+	+
151		+	-	+	+	+	+	e	+	+	++	-	-

TABLE X (continued)

*C#	O IN T	Mot	Pig Prod	Temperature Relationships Growth at 0° C	4-6° C	32° C	37° C	A L M	Liq Gel	Red Nit	Cab Met Fer	Cab Met Oxi	Butterfat Hydrolysis
219	5° C 15 days	+	-	+	+	+	+	e	+	+	-	+	+

*KEY:

Column Captions

C# - Culture number
O IN T - Original incubation temperature
Mot - Motility
Pig Prod - Pigment production
A L M - Action on litmus milk
Liq Gel - Liquefaction of gelatin
Red Nit - Reduction of nitrates
Cab Met - Carbohydrate metabolism
Fer - Fermemtation
Oxi - Oxidation

Table Interior

a - Alkaline reaction
b - Alkaline and reduction
c - Acid and reduction
d - Acid, reduction, and coagulation
e - Only reduction
+ - Positive
- - Negative
± - Weak

15, 59 - Gram-Positive Rods Predominant and some Gram-Negative Rods present.

56, 141 - Gram-Negative Rods Predominant and some Gram-Positive cocci present

All Others - Gram-Negative Rods Predominant and some Gram-Positive Rods present.